REMARKS

Reconsideration of the above-identified application in view of the amendments above and the remarks following is respectfully requested.

Claims 1- 47 and 62-81 are in this case. Claims 1- 47 and 62-81 have been rejected. Claims 1, 5, 8, 12, 14, 19, 23, 24, 29, 33 and 34 have now been amended. Claims 9, 10, 21, 21, 30, 31, 40, 41 and 62-81 have now been cancelled. New claims 84-91 have been added.

Claim Objections

The Examiner has objected to the word "port" in claim 40, line 2. Claim 40 has now been cancelled, rendering moot the Examiner's objection thereto.

35 U.S.C. § 112, First Paragraph, Rejections

The Examiner has rejected claims 1-47 and 62-81 under U.S.C. 112, first paragraph as containing subject matter that was not described in specification in such a manner as to reasonably convey to one skilled in the art that the inventors had possession of the claimed invention at the time of filing. The Examiner's rejections are respectfully traversed. Claims 9, 10, 21, 21, 30, 31, 40, 41 and 62-81 have now been cancelled, rendering the Examiner's rejections moot with respect to these claims. Claims 1, 5, 8, 12, 14, 19, 23, 24, 29, 33 and 34 have now been amended.

In particular, the Examiner states that while the instant specification describes an isolated polynucleotide from *Citrus* encoding a polypeptide having a flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase catalytic activity, and an isolated polynucleotide of SEQ ID NO: 20 from pummelo encoding a flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase of SEQ ID NO: 21, no other polynucleotides encoding a flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase from any species other than pummelo, or any polynucleotide sequence encoding a polypeptide having between 50- 100%

sequence identity to SEQ ID NO: 21, or any nucleotide sequence having 50-100% sequence identity to SEQ ID NO: 20 or any functional or active parts thereof other than SEQ ID NO: 20, or any nucleotide sequence encoding or expressing an RNA molecule that will *in vivo* base pair with, or *in vitro* hybridize to SEQ ID NO: 20 or any naturally occurring polynucleotide sequence encoding a flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase, as recited in the claims, are described. Thus, the Examiner concludes that the specification does not provide an adequate written description of the claimed invention, in view of the claim breadth and "lack of guidance". The Examiner further states that cells and plants transformed with the polynucleotide as claimed are also inadequately described.

The present invention is of a 1-2-rhamnosyl-transferase gene and the recombinant protein product thereof (SEQ ID NO: 21), which can be used in a multistep process of converting hesperidin from orange peels to the sweetener neohesperidin dihydrochalcone, and in providing genetically modified plants of the genus *Citrus* having reduced bitterness. While reducing the present invention to practice, a polynucleotide encoding a polypeptide having unique flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase activity was isolated and cloned from Pummelo young leaf mRNA by RT-PCR using unique gene specific PCR primers containing sequences based on partial peptide sequencing of the purified flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase from Pummelo, and completion of the full length cDNA sequence using 5'RACE with gene specific primers. As described in the instant specification, the isolation and cloning of the full length flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase cDNA was unattainable using traditional screening of cDNA libraries with either oligonucleotide probes or antibodies. Further, although the glucosyl transferase family is large, and several glucosyl transferases catalyzing the transfer of UDP-sugar to flavonoids have been described, the specific flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase (α-1-2 rhamnosyl transferase) activity of the

polypeptide encoded by the polynucleotides of the present invention has not been demonstrated for any other known sequences. Thus, the coding sequence of the present invention is unique to this specific enzyme.

Hence, although portions of the polynucleotide of the present invention may exhibit shared homologies with coding sequences of other members of the glucosyl transferase superfamily, none of the partially homologous sequences "encodes a polypeptide having a flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase activity...", as recited in the instant specification and claims. Applicant wishes to point out that the Written Description Requirement, as defined in the guidelines, need not provide description of all possible species of a claimed genus but rather merely identify characteristics of a species in a manner which illustrates to the ordinary skilled artisan that the inventor was in possession of the invention at the time of filing. Thus, one of ordinary skill in the art, in possession of the teachings of the present invention, would have a reasonably high expectation of identifying and isolating all polynucleotides having both high sequence homology to SEQ ID NO: 20, and encoding a polypeptide having a flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase activity using techniques of hybridization and enzyme assay as described in the instant specification,(see specific recitation of methods of "identifying and cloning a flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase homologue from plant species other than pummelo", page 11, lines 13-38) or, for example, in *Short Protocols in Molecular Biology,* Second Edition, Ausabel et al. ed, John Wiley and Sons, 1992.

Thus, it is Applicant's strong opinion that the instant specification provides an adequate written description of the function and structural features common to polynucleotides having a high degree of homology in SEQ ID NO: 20, encoding a polypeptide having a flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase. That notwithstanding, and to further define the claims of the present invention and to expedite prosecution of this case, independent claims 1, 14, 24 and 34 have now been amended to recite: "...having at least

80% sequence identity with SEQ ID NO: 20 as determined using a sequence analysis software package developed by the Genetic Computer Group (GCG) at the University of Wisconsin with gap creation penalty of 50 and gap extension penalty of 3, and/or hybridizable with SEQ ID NO:20 under conditions of stringent hybridization, wherein said stringent hybridization is effected by a hybridization solution of 6 x SSC and 1 % SDS, hybridization temperature of 65 °C, final wash solution of 0.1 x SSC and final wash at 60 ° C, the isolated polynucleotide encoding a polypeptide having a flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase catalytic activity.", thus now limiting the polynucleotide of the invention to those having both an extremely high degree of homology, by sequence analysis an/or stringent hybridization, and encoding a polypeptide having a flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase catalytic activity. Similarly, dependent claim 12 has now been amended to recite "The isolated polynucleotide of claim 1, wherein said polypeptide shares at least 75% identical or conserved amino acids with SEQ ID NO:21, as determined using a sequence analysis software package developed by the Genetic Computer Group (GCG) at the University of Wisconsin with gap creation penalty of 12 and gap extension penalty of 4", limiting the polypeptide encoded by the polynucleotide of the invention to at least 75% identity with the Pummelo enzyme.

Further limitations have been provided by the removal of the phrase "...or a functional part thereof." from now amended claims 8, and 12 and the phrase "or a portion thereof longer than 15 nucleotides" from now amended claims 19, and 29.

Thus, independent claims 1, 14, 24 and 34, and all claims directly or indirectly depending therefrom, no longer relate to a polynucleotide sequence encoding a polypeptide having between 50-100% sequence identity to SEQ ID NO: 20 or any functional parts or active parts thereof, or any nucleotide encoding or expressing an RNA molecule that will *in vivo* base pair with, or *in vitro* hybridize to SEQ ID NO: 20 or any other naturally occurring

polynucleotide sequence encoding a flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase, rather to polynucleotides having high sequence homology with the full length cDNA of the invention and encoding a functional polypeptide having a flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase catalytic activity.

Support for these amendments can be found throughout the instant specification, for example, page 9, line 25-34 (percent homology of nucleotide by sequence analysis), page 9, line 35 to page 10, line 11 (stringent hybridization conditions), page 10, line 12-24 (polypeptide homology by sequence analysis), and page 12, lines 1-26 (nucleic acid and polypeptide homology).

The Examiner has further rejected claims 1-47 and 62-81under 35 USC § 112, first paragraph, because the specification does not enable any person skilled in the art to make and use the invention commensurate with the claims. The Examiner's rejections are respectfully traversed. Claims 9, 10, 20, 21, 30, 31, 40, 41 and 62-81 have now been cancelled, rendering the Examiner's rejections moot with respect to these claims. Claims 1, 5, 8, 12, 14, 19, 23, 24, 29, 33 and 34 have now been amended.

The Examiner states that although the instant specification teaches the isolation of a polypeptide from pummelo having flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase activity by means of partial peptide sequencing to obtain primers for RT and RACE PCR, the isolation the 5" end of the pummelo cDNA clone encoding flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase , and the polynucleotide of SEQ ID NO: 21, a flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase from pummelo, the instant specification fails to teach <u>any</u> nucleotide sequences encoding a flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase or fragments or active parts thereof other than SEQ ID NO: 20, or plants or cells transformed therewith.

The Examiner further states that stringent hybridization conditions do not always select for DNA having identical or nearly identical nucleotide

sequence as the probe, and that the phenotypic character expected from expression of a DNA construct cannot always be reliably predicted. Thus, the Examiner concludes that one of ordinary skill in the art would be required to engage in undue trial and error experimentation to isolate the "multitude of non-exemplified sequences encoding a flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase..."

Applicant wishes to point out that the present invention discloses, for the first time, a nucleotide sequence encoding a polypeptide having flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase activity. While reducing the present invention to practice, the inventors have cloned and sequenced the full length coding sequence of flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase, from pummelo, which activity has not been demonstrated for any other known sequences (see above). Thus, expressible polynucleotides having a high degree of sequence homology to, for example, SEQ ID NO:20 of the instant specification, can be expected, with reasonable reliability, to encode a polypeptide having flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase activity.

Examiner states that the phenotypic character resulting from expression of a DNA construct is often unpredictable, and thus concludes that techniques for modulation of gene expression well known in the art such as antisense and co-suppression require undue experimentation for their application. Applicant wishes to point out that examples of successful manipulation of availability of specific gene transcripts abound, such as the use of antisense oligonucleotides for the treatment of CMV eye infections (Lyngstadass A, Int J Tech Assess Health Care 2002,18:645-74), Crohn's disease (Yacyshyn et al., Aliment Pharm Ther 2002; 16:1761-70), prostate and other cancers. Indeed, the Examiner has confirmed the accessibility of the antisense methodology recited in the present invention, stating in section 8 , page 11 of the present Office Action, that "One would have been motivated by the teachings of Luth (transgenic grapefruit plants, 1999) that the method was generally applicable

using any nucleotide coding sequence or fragment or portion thereof. One would have had a reasonable expectation of success of modifying the levels of flavanone-7-*O*-glucoside-2"-O-rhamnosyl transferase activity of grapefruit in view of the success of Luth. Choice of intra or extra chromosomal integration would have been an optimization of experimental parameters within the skill level of the ordinary artisan."

In order to further define the claims of the present invention, and to expedite prosecution of this case, independent claims 1, 14, 24 and 34 have now been amended to recite: "...having at least 80% sequence identity with SEQ ID NO: 20 as determined using a sequence analysis software package developed by the Genetic Computer Group (GCG) at the University of Wisconsin with gap creation penalty of 50 and gap extension penalty of 3, and/or hybridizable with SEQ ID NO:20 under conditions of stringent hybridization, wherein said stringent hybridization is effected by a hybridization solution of 6 x SSC and 1 % SDS, hybridization temperature of 65 °C, final wash solution of 0.1 x SSC and final wash at 60 °C, the isolated polynucleotide encoding a polypeptide having a flavanone-7-*O*-glucoside-2"-*O*-rhamnosyl-transferase catalytic activity.", thus now limiting the polynucleotide of the invention to those having both an extremely high degree of homology, by sequence analysis and/or stringent hybridization, and encoding a polypeptide having a flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase catalytic activity. Similarly, dependent claim 12 has now been amended to recite "The isolated polynucleotide of claim 1, wherein said polypeptide shares at least 75% identical or conserved amino acids with SEQ ID NO:21, as determined using a sequence analysis software package developed by the Genetic Computer Group (GCG) at the University of Wisconsin with gap creation penalty of 12 and gap extension penalty of 4", limiting the polypeptide encoded by the polynucleotide of the invention to at least 75% identity with the Pummelo enzyme.

Further limitations have been provided by the removal of the phrase "...or a functional part thereof." from now amended claims 8, and 12 and the phrase "or a portion thereof longer than 15 nucleotides" from now amended claims 19, and 29.

Thus, independent claims 1, 14, 24 and 34, and all claims directly or indirectly depending therefrom, no longer relate to a "multitude of nucleotide sequences" or fragments, as stated by the Examiner, rather to polynucleotides having high sequence homology with the full length cDNA of the invention, and further characterized by being capable of encoding a functional polypeptide having a flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase catalytic activity. Support for such amendments is found throughout the instant specification, as detailed hereinabove.

In view of the above, it is Applicant's strong opinion that one of ordinary skill in the art, privileged to the teachings of the present invention, and employing any of the well known techniques described herein, could make and use the constructs and methods of the present invention to identify and/or generate nucleotide sequences encoding a flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase and plants or cells transformed therewith having modified flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase activity without engaging in undue experimentation.

In view of the arguments and claims amendments indicated hereinabove, Applicant believes to have overcome all 35 U.S.C. § 112, first paragraph rejections.

35 U.S.C. § 112, Second Paragraph, Rejections

The Examiner has rejected claims 10, 21, 31, 41, 69 and 79 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter that Applicant regards as the invention. Claims 10, 21, 31, 41, 69 and 79 have now been cancelled, rendering the Examiner's rejections moot with respect to these claims.

35 U.S.C. § 103 (a) Rejections- Luth, D et al. in view of Mok, D., et al. and Bar-Peled M, et al.

The Examiner has rejected claims 14-18, 20-21, 24-28, 30-31, 62-69 and 72-79 under 35 U.S.C. § 103 (a) as being anticipated by Luth D, et al. in view of Mok. D, et al. and Bar-Peled, et al. Claims 20, 21, 30, 31, 62-69 and 72-79 have now been cancelled, rendering the Examiner's rejections with regard to these claims moot. Claim 14, from which claims 15-18 directly depend, and claim 24, from which claims 25-28 directly depend, have been amended. The Examiner's rejections are respectfully traversed.

The Examiner states that Luth et al. teaches transformation of grapefruit, that Mok et al. teaches the isolation of a polynucleotide having sequence homology to portions of SEQ ID NO: 20, and that Bar-Peled et al. teach the isolation of a flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase activity from grapefruit, and it's usefulness in genetic engineering to modify flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase activity. Thus, the Examiner concludes that it would have been prima facie obvious to one of ordinary skill in the art to modify the invention of Luth et al. in view of the nucleotide sequence taught by Mok et al. and the utility of modifying bitterness taught by Bar-Peled et al. with a reasonable degree of success.

The prior art sequence taught by Mok et al. (GenBank Accession No. AF101972) is of a Phaseolus lunatus zeatin o-glucosyltransferase (ZOG1) mRNA. The prior art sequence, like many other sequences of the UDP-glucosyl transferase superfamily, includes domains conferring glucosyl-transferase catalytic activity common to all members of this superfamily. As a result, there is an element of homology between the prior art sequence and the nucleotide sequence of the present invention. However, the ZOG1 taught by Mok et al. is specific for the transfer of a glucose or xylose moiety from an activated saccharide to the substrate zeatin, and is devoid of flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase activity. This is reflected in the limited degree of homology uncovered between the nucleotide sequences (maximum

approx 60% homology for a sequence representing 60% of the coding sequence of flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase, by LALIGN (ISREC, gap penalty –14/-4).

Further, as stated hereinabove, and noted in the instant specification isolation and cloning of the nucleotide sequence of the present invention required extensive experimentation, following the failure of common techniques such as polyclonal antibody screening, and oligonucleotide screening of conventional cDNA or expression libraries to yield positive clones (see Examples, pages 22-24). In view of these results, it is unlikely that specific sequences having high degree of homology to the flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase coding sequence uncovered by the present inventors could have been identified and isolated using the prior art sequence of Mok et al.

Similarly, the prior art reference of Bar-Peled et al. (J Biol Chem, 1991;266:20953-59) reports the isolation of UDP-rhamnose:flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase from citrus, it's physical and kinetic characterization, and the purification thereof. The authors make the extremely general and non-committal statement, at the end of the reference, that "the isolation of the gene may enable it's use in genetic engineering directed to modifying grapefruit bitterness", however, no mention is made of methods for isolation of the nucleotide sequence. Indeed, many years of effort were required before the isolation of the coding sequence of the present invention was made possible by the present inventors. Thus, the reference of Bar-Peled et al. would not provide motivation to one of ordinary skill in the art to make or use the nucleotide sequence encoding flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase of the present invention with a reasonable expectation of success.

The Examiner has stated that Luth et al. teach the transformation of grapefruit. Luth et al. report the transformation of grapefruit with the scorable marker beta-glucoronidase and the selectable marker NPTII, and the

regeneration of transgenic shoots from epicotyl segments. However, there is no mention of transformation with antisense constructs, or of modification of levels of flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase in transformed plant cells.

Thus, the prior art sequence of Mok et al., in view of Luth et al. and Bar-Peled et al., neither teaches nor motivates nor anticipates the modifying of bitterness in grapefruit by modifying flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase activity.

The abovementioned notwithstanding, and in order to further define the claims of the present invention, and expedite prosecution of this case, independent claims 14 and 24 have now been amended to recite: "...having at least 80% sequence identity with SEQ ID NO: 20 as determined using a sequence analysis software package developed by the Genetic Computer Group (GCG) at the University of Wisconsin with gap creation penalty of 50 and gap extension penalty of 3, and/or hybridizable with SEQ ID NO:20 under conditions of stringent hybridization, wherein said stringent hybridization is effected by a hybridization solution of 6 x SSC and 1 % SDS, hybridization temperature of 65 °C, final wash solution of 0.1 x SSC and final wash at 60 ° C, said polynucleotide designed encoding nucleotide sequences complementary to, and capable of binding to flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase transcripts, and whereas expression of said expressible polynucleotide decreases the level of flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase catalytic activity in said plant cell.". Thus, independent claims 14 and 24, and all claims directly or indirectly depending therefrom, no longer relate to any nucleotide sequence encoding an antisense RNA molecule being capable of in vivo base pairing with flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase mRNA, but now relate exclusively to polynucleotides having both an extremely high degree of homology, by sequence analysis and/or stringent hybridization, and designed capable of decreasing levels of

flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase activity in the transformed cell.

Thus, it is Applicant's strong opinion that, despite the limited similarity of the prior art zeatin transferase sequence disclosed by Mok et al. to the flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase nucleotide sequence of the present invention, the methods and transformed plants having modified flavanone-7-O-glucoside-2"-O-rhamnosyl-transferase activity of the present invention are novel and non-obvious, and, contrary to the Examiner's conclusion, one of ordinary skill in the art could not have been motivated with a reasonable expectation of success, to make or use the methods and transformed plants of the present invention by Mok et al. in view of the teachings of Luth et al. and Bar-Peled et al.

New Claims

New claims 84-91 have been added. No new subject matter has been added to the new claims.

In view of the above amendments and remarks it is respectfully submitted that claims 1-8, 12-19, 22-29, 32-38, and new claims 84-91 are in condition for allowance. Prompt notice of allowance is respectfully and earnestly solicited.

Respectfully submitted,

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Date: June 1, 2003.

Encl.:
A three months extension fee.